being highly expressed; the apparent discrepancy between our data and those reported by Hilmi *et al*. may be due to one of several factors. First, the authors used only two cell lines in their studies, which might have not been enough lines to accurately predict a similar association across a larger sample size. Next, our gene expression profiling was performed in the continued presence of excess (5 μg/ml) insulin, rather than a transient treatment with IGF-1 to serum-starved cells; this minor experimental difference could account for the discrepancy between the two sets of data.

Although the results of Hilmi *et al*. (2008) help to establish a role for IGF-1 signaling in expression of anti-apoptotic molecules, they leave other questions unanswered. For example, what are the molecular mechanisms by which IGF-1 is able to induce expression of Bcl-2, Bcl-X_L, and survivin—are these proteins coregulated or similarly affected by distinct downstream mediators of the IGF-1 signaling axis? Likewise, MeWo and A375 cells represent metastatic melanomas—is the prosurvival phenotype after IGF-1 treatment a function of disease stage or is this observation merely coincidental? Furthermore, does inhibition of IGF-1 signaling (via IGF-1R monoclonal antibodies, for example) synergize with traditional chemotherapeutics to initiate disease regression? These types of studies appear promising in preclinical investigations (Ji *et al.*, 2007; Maloney *et al.*, 2003), whereas results from early clinical trials are still pending.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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First-Class Delivery: Getting Growth Factors to Their Destination

Jeffrey M. Davidson[1]

Growth factor bioavailability in therapeutic applications such as wound healing is limited by extracellular matrix sequestration, proteolysis, and clearance. Local, transient delivery by gene transfer is an attractive concept. Many transfection strategies are available, and adenoviral vectors are in clinical trials. Keratinocyte growth factor-1 (KGF-1), an epithelial-specific member of the fibroblast growth factor (FGF) family, has achieved limited success in protein formulations. Matrix- and cell-based strategies for delivering a KGF-1 virion to target tissue may improve the reproducibility and efficiency of the process, although the advantages of cell-based therapy must be weighed against its added cost and complexity.

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Despite two decades of advancement in the understanding and use of growth factors for wound healing, there has been limited clinical success (Leahy and Lawrence, 2007; Papanas and Maltezos, 2007). Numerous strategies for transient gene delivery of growth factor cDNA, by driving sustained, local overexpression of the factor, appear to overcome obstacles to delivery of and response to these proteins in the hostile wound environment (Eming *et al.*, 2007). Transient gene delivery avoids many of the challenges of stable transformation needed to correct genetic defects. Among the many potential approaches for DNA transfer, early clinical findings with adenoviral recombinant platelet-derived growth factor-BB (PDGF-BB) therapy have been promising. Using a humanized mouse model, Escámez *et al*. (2008, this issue) have compared several methods

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of expressing adenovirally delivered FGF-7/KGF-1 in small excisional wounds with a principal end point of wound resurfacing. They report that fibrin gels containing either free virus or virally transformed fibroblasts yield more consistent improvement in epidermal closure than intradermal injection of free adenovirus.

The authors previously described a useful wound model in which human living skin equivalents (LSEs) were implanted in immunodeficient mice (Escámez *et al.*, 2004). This approach has the advantage of standardization, analysis of human cells in a tissue construct, and a reduced wound contraction artifact, with the limitation that the graft develops as a chimera of mouse vascular and hematopoietic derivatives with defined human cutaneous cell populations. As a positive control for growth factor efficacy, the present study illustrates that an LSE containing keratinocytes that have been stably transformed by a retroviral KGF-1 vector demonstrated more rapid closure. The potential drawback of sustained KGF-1 delivery is evident from the hypertrophic epidermis as shown in Figure 1 of the authors' article in this issue.

KGF-1 is an interesting choice for this study because it is one of the clearest examples of paracrine interaction between the expressing fibroblast and the responding epidermal cell (Werner *et al.*, 2007). Effective growth factor action requires short distances between the secreting and target cells, and members of the FGF family tend to bind quite strongly to heparan sulfate proteoglycans in the interposing extracellular matrix. KGF-1 presents several useful characteristics of a vulnerary agent, including enhanced epithelial differentiation and protection against oxidative stress. The main application for KGF-1 has been directed toward oral mucositis (Radtke and Kolesar, 2005; Siddiqui and Wellington, 2005); however, the development of KGF-2/FGF-10 for chronic wounds has not proceeded beyond earlier trials on wound healing (Robson *et al.*, 2001). Topical application of growth factors, at least in simple delivery vehicles, seems to be a relatively ineffective method of drug delivery. Wound healing effects with rhPDGF-BB require industrial quantities of protein with potential systemic effects (Steed, 2006), whereas gene delivery is more efficient by several orders of magnitude (Gu *et al.*, 2004).

Cell-based wound healing strategies; candidates for genetic manipulation

Escámez *et al.* observed that peri-wound injection of free adenovirus expressing KGF-1 was effective in enhancing wound resurfacing, but the outcome was extremely variable. On average, epidermal proliferation was nonetheless similar to the other delivery models. KGF activity, in terms of cell migration, may be particularly sensitive to the distance between the viral target and the KGF target. To obtain more consistent effects, the authors then compared free virus in a fibrin gel overlay with virally transformed fibroblasts in a fibrin gel. Both of these methods produced more consistent findings, although there was not a dramatic improvement in efficacy. Others have advocated a collagen-based biodegradable vehicle, which is likely to be more persistent (Doukas *et al.*, 2001). This study did not address the potential effect of the fibrin overlay alone, nor did it include a direct comparison with rhKGF-1 protein, although the authors' previous publication reported similar effects for injecting 1.5 μg of the protein on the first 3 days after surgery. According to ELISA data in the article in this issue, adenoviral gene transfer produced biological effects at 5- to 10-fold lower steady-state tissue levels of KGF-1, consistent with the enhanced efficacy offered by local transgene expression.

Do these findings support a more practical and (cost-) effective means of realizing the potential of growth factors? The authors suggest that both gel-mediated methods are superior—at least in terms of reproducibility—to simple, peri-wound injection. They further suggest that the cell-based approach can have higher efficiency because of the higher KGF-1 levels achieved, although the outcomes of each treatment in terms of closure and proliferation were indistinguishable. Current clinical trials with adenoviral PDGF-BB utilize free adenovirus in a collagen gel matrix that is applied to the wound surface (Gu *et al.*, 2004) or injected into the wound margin (Margolis *et al.*, 2000, 2004). The practical advantages of a matrix-based delivery system are convenience, persistence, and adhesion to the wound site. Injection, on the other hand, can target deeper tissues; however, because KGFs exclusively bind to epithelial receptors, the expressed gene product may fail to diffuse to its target. Deeper infiltration may not be a disadvantage for treatment of the dermis with other growth factor vectors. The cell-based system is more sophisticated and has the potential advantage of standardizing the amount of growth factor produced because the cells would be infected with virus *in vitro*. Cell-based therapies for burn and wound treatments are widely practiced, and at least three separate LSE technologies have been commercially developed. Thus, this report is a useful proof of principle that exogenous cells can be applied to wounds and used to stimulate repair through either endogenous or transduced expression of healing agents.

Escámez *et al.* (2008) created the cellular system by infecting, washing, and incubating cells and transplanting them to the wound site. This would be a complex and costly approach and not easily scaled or transported to the clinic. If autologous fibroblasts were used in this application, additional delay would be imposed by the expansion of donor cell populations. The transient expression of adenoviral genes may make it difficult to execute this therapy, except at facilities that are equipped to manage cell culture for human therapy. The use of allogeneic cells with stable or regulated transgene expression would be a more promising tactic.

Cell-based wound healing strategies have used autologous or allogeneic sources of keratinocytes, fibroblasts, mesenchymal stem cells, and unfractionated marrow. Tissue equivalents, containment devices, and

cell suspensions in biological matrices have been used to immobilize the cells and to permit interactions with the host tissue (Lanza *et al.*, 2007). There is very little evidence that these devices—at least in the wound environment—lead to integration or long-term survival, yet there is not compelling evidence for rejection. The presumed mode of action for an LSE is paracrine stimulation, perhaps in response to the wound environment. It has been obvious for some time that these cell-based cutaneous treatments are prime candidates for genetic manipulation if safety and efficacy issues can be satisfactorily resolved. However, it is not certain that the added costs of production and safety measures can yield a cost-effective drug delivery system. Cell systems that are engineered to deliberately express one or more biologicals would fall under more stringent regulatory scrutiny. One can envisage a strategy in which cell-based healing devices are customized to deliver various doses of various factors, including proteinase inhibitors, depending on the type of wound and the stage of healing. The effects seen in this model system, which uses a human skin equivalent as opposed to intact human tissue, point in a positive direction.

CONFLICT OF INTEREST
The author states no conflict of interest.

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Filaggrin Mutations and Allergic Contact Sensitization

Jon M. Hanifin[1]

In this issue, Novak *et al.* (2008) provide evidence that *filaggrin* barrier defects might also predispose to allergic contact dermatitis by allowing greater penetration of chemical haptens. Their report provides a fresh perspective on the issues of contact allergy, nickel sensitization, and stratum corneum defects.

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The eczemas comprise a family of inflammatory skin diseases that have as hallmarks itch, epidermal spongiosis, and disruption of the stratum corneum barrier. Atopic dermatitis (AD) and allergic contact dermatitis (ACD) are among the most common and widely studied of the eczemas. In the spring of 2006 a revealing new light focused on AD, firmly associating that disease with ichthyosis vulgaris and loss-of-function mutations in the *filaggrin* (*FLG*) gene (Palmer *et al.*, 2006), and subsequent studies have confirmed that finding (Irvine, 2007). This insight gave molecular support to long-standing predictions that AD might be caused by an epidermal barrier defect allowing penetration of irritants, microbes, and protein antigens (Wood *et al.*, 1992).

Those revelations led naturally to the question of whether FLG barrier defects might also predispose to ACD by allowing greater penetration of chemical haptens. In this issue, Novak and collaborators in Germany provide evidence that the answer may be yes (Novak *et al.*, 2008). These investigators looked for two common *FLG* mutations in a cross-sectional population that had been studied and patch tested with common chemicals in the KORA Allergy Study from 1994 to 1995 (Schäfer *et al.*, 2001). They selected

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